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VARIABILITY IN THE DIPHTHERIA GROUP OF BACILLI.*

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ONE of the most interesting questions in bacteriology at present is that of the mutual relationship borne by the organisms in the so-called "groups." In the case of the diphtheria group the problem is of particular importance because of its bearing on public health service methods, as these naturally must differ in many respects if the group consists of two or more distinct species instead of but one comprised of several types.

The overwhelming majority of bacteriologists at present look upon the group as consisting of at least three definite species, *B. diphtheriae* (Klebs-Löffler[1]), *B. pseudodiphthericus* (Hofmann-Wellenhof[2]), and *B. xerosis* (Kuschbert and Neisser[3]), while opinion is divided in regard to the numerous other types, collectively known as diphtheroids, of which the most important are *B. coryzae segmentosus* (Cautley[4]), *B. diphtheroides* (Klein[5]), *B. clavatus* (Kruse and Pasquale[6]), *B. septatus* (Gelpke[7]), and *Corynebacterium lymphae vaccinae* (Levy and Fickler[8]). Some investigators consider all these forms as varieties of either *B. pseudodiphthericus* or *B. xerosis*, while others make separate species of some or all. On the other hand, Roux and Yersin[9] and, more recently, Behring[10] look upon the diphtheria group as but a single species, regarding the morphological and biological differences between the types as insufficient to justify establishing new species. Their views, however, have found few adherents.

The important features of typical examples of the three main forms may be summarized as follows:

MORPHOLOGY.

B. diphtheriae: Long, slender rods, straight or slightly bent.
B. pseudodiphthericus: Short, plump, straight rods.
B. xerosis: Similar to *B. diphtheriae.*

NEISSER STAIN.

B. diphtheriae: Babes-Ernst granules demonstrable.
B. pseudodiphthericus: Solid-staining.
B. xerosis: Granules, if present, appear late (after 24 hours).

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Löffler's Inspissated Serum.

B. diphtheriae: Growth is rapid and luxuriant. Vigorous development occurs during the first 12 hours and the maximum is practically reached in 24 hours. Consistency of the growth is firm and coherent and its surface dull and grayish in appearance.

B. pseudodiphthericus: Growth is meager. Development during the first 12 hours is slow, and at 24 hours appreciably less than that of the Klebs-Löffler bacillus. The consistency is soft and even viscid. The surface is moist, smooth, lustrous, and pure white.

B. xerosis: Growth is very scanty. No development is visible until after 24 hours. The consistency resembles that of *B. diphtheriae*. The surface is dull, gray, and extremely dry.

Agar.

B. diphtheriae: Growth is slow and meager. Colonies are small, delicate, and transparent with a dull, gray surface.

B. pseudodiphthericus: Growth is rapid and luxuriant. Colonies are white, moist, lustrous, and spreading.

B. xerosis: Very scanty growth, frequently invisible for two or even three days. Colonies are very small and transparent and present a dull surface.

Pathogenicity.

B. diphtheriae: In guinea-pigs causes edema, petechiae, or larger hemorrhages, and a fibrinous exudate at the site of subcutaneous inoculation. The adrenals are enlarged and hemorrhagic and frequently the lymph-glands are likewise. More or less serous exudate is found in the peritoneal cavity and sometimes in the pleural cavities as well. The animal either dies acutely of toxemia or gradually wastes away and dies of cachexia weeks afterward, according to the degree of virulence of the organism. The rabbit is equally susceptible but the lesions are not so constant.

B. pseudodiphthericus and *B. xerosis* are non-pathogenic.

Soluble Toxin Production.

Demonstrable only with *B. diphtheriae*.

Acid Production in Carbohydrate Broth.

B. diphtheriae: Dextrose (and dextrin).

B. pseudodiphthericus: None.

B. xerosis: Dextrose (and saccharose).

Apparently the differences exhibited are pronounced enough to warrant acceptance of these three forms, at least, as distinct species. The existence of definite and significant points of difference, however, is but one of the two requisites for this recognition, the other being permanence of the characters, and while the first is established, the latter is not beyond question. This can best be shown, perhaps, by taking each character separately and reviewing chronologically the more salient researches thereon.

Morphology.—Zarniko,[11] as early as 1889, reported that he was unable to correlate the pathogenicity with the morphology of diphtheria cultures.

Peters[12] found two types of pathogenic diphtheria bacilli which differed morphologically and remained distinct after two years of cultivation except that the shorter approached the pseudodiphtheria type and became avirulent. Nevertheless he does not regard *B. pseudodiphthericus* as an attenuated *B. diphtheriae.*

Ferré and Creignou[13] and Gouguenheim and Dutertre[14] could establish no relation between morphology and virulence.

Kurth[15] believes that the Klebs-Löffler and the Hofmann-Wellenhof organisms can be distinguished by merely noting the length of the bacilli.

De Simoni[16] found a spore-bearing organism of the pseudodiphtheria morphology in ozena secretion.

Wesbrook, Wilson, and McDaniel[17] described three principal (granular, barred, solid-staining) and some 28 secondary forms of diphtheria bacilli. They report that in pure cultures from typical clinical cases of diphtheria there is never complete uniformity in the shape, size, and staining reactions of the individual bacilli and that even a moderate degree of uniformity is exceptional. It appears probable, therefore, that the metamorphosis of the classical *B. diphtheriae* into the solid-staining pseudodiphtheria type occurs rather readily in the throat and that the converse may occur.

Gorham[18] corroborates the above as to the change of the granular to the solid-staining type during convalescence. He considers this due to the production of immune bodies and that it is accompanied by loss of virulence.

Macé[19] found no relation between morphology and virulence in cultures he examined.

Lesieur[20] found that some bacilli of the pseudodiphtheria type may be highly virulent. By animal passage in collodion sacs through three rabbits he found that Hofmann-Wellenhof forms took on the morphology of *B. diphtheriae,* while on the other hand by cultivation for eight months in diffuse light he changed three diphtheria strains to forms indistinguishable from *B. pseudodiphthericus.*

Ohlmacher[21] similarly changed the morphology of a short, solid form to the slender, curved type and a granular to the short, pseudodiphtheria appearance (though it still formed acid) by means of animal passage.

Denny[22] made a very careful study of the morphology of serum cultures at intervals during the first 12 hours of growth. In the case of 10 Klebs-Löffler strains he found that they first appeared as solid-staining forms which later changed to the granular type and exhibited elongated forms, swollen ends, and tinctorial segmentation. He found that unfavorable conditions of growth and symbiosis with other bacteria generally delayed the development of the granular forms and suggests that symbiosis (e. g., with streptococci) may account for the change to the solid type during convalescence. Thirty-five strains of *B. pseudodiphthericus* always presented the short form, while eight strains of *B. xerosis* passed through the same stages as the Klebs-Löffler, although usually they were shorter and thicker than the latter. He believes, nevertheless, that the three types can be regarded as separate species and distinguished from one another if all the stages of their development are considered.

Hamilton[23] isolated some strains from scarlatina, measles, and diphtheria morphologically identical with the Klebs-Löffler bacilli but which were regarded as *B. pseudodiphthericus* on the ground of serum reactions and of pathogenicity.

Hadley[24] reports transforming the granular into the solid-staining type and *vice-versa* by passage through guinea-pigs.

Babes-Ernst granules.—Babes[25] was probably the first to see the characteristic granules in cultures of diphtheria bacilli. He thought them spores at the time.

Ernst[26] about the same time reported seeing the granules in *B. xerosis.*

Cronch,[27] who stained the granules with 1 per cent solution of methyl green, observed that they appeared in all the strains of the group but considerably earlier in the Klebs-Löffler bacillus than in the related types. He regarded this as characteristic of *B. diphtheriae.*

In 1897 M. Neisser[28] published the method, now universally employed, for staining the granules in the diphtheria bacillus. He offered it as a reliable means for distinguishing between the true and the false diphtheria forms, although admitting that the granules were present occasionally in *B. pseudodiphthericus,* though never in *B. xerosis.* They appeared between the ninth and twenty-fourth hours.

Heinersdorff[29] found the granules in *B. diphtheriae* appeared between the eighth and twelfth hours, while those he found in *B. xerosis* were not visible until after 24 hours. He examined 60 strains of *B. xerosis* of less than 12 hours growth but could not find typical granules in any.

Kurth[15] and also Gramakowsky[30] found the granules present in *B. pseudodiphthericus.*

Schanz[31] found the Babes-Ernst granules in *B. xerosis,* although later than in *B. diphtheriae.*

Cobbett[32] reported finding no granules in 69 strains of *B. pseudodiphthericus,* using Neisser's stain.

Lesieur[20] found the granules in 80 per cent of the diphtheria and in 20 per cent of the pseudodiphtheria strains examined. He could ascertain no constancy in the time of their appearance. Prolonged cultivation in diffuse light caused the granules to disappear.

Denny[22] states that in contradistinction to the granules of *B. diphtheriae,* those of *B. xerosis* stain readily with Löffler's methylene blue but poorly with the Neisser granule stain. He found granules present also in *B. pseudodiphthericus,* though but rarely.

Gordon[33] observed the granules in *B. coryzae segmentosus* and in four other diphtheroids.

Graham-Smith[34] was able to demonstrate these structures in a considerable number of diphtheroid "species."

Pathogenicity.—Löffler[35] noted that *B. diphtheriae* in cultures loses its virulence after a time without any concomitant change in cultural characters.

Roux and Yersin,[9] by making throat cultures at various stages after the termination of the disease, discovered that the bacilli in the mouth gradually became attenuated. Virulent diphtheria bacilli, cultivated at 39.5° C. or in a current of air, became attenuated and approached the characters of *B. pseudodiphthericus,* while Klebs-Löffler bacilli of low virulence could be made highly pathogenic by passage through a guinea-pig, in symbiosis with the streptococcus of erysipelas. The experiment did not succeed, however, when totally avirulent bacilli were used.

Escherich,[36] Goldscheider,[37] Sudeck,[38] and Zupnik[39] were unable to confer virulence on strains of *B. pseudodiphthericus.*

Kruse and Pasquale[6] cultivated a diphtheroid form, which they call *B. clavatus,* from a liver abscess in amebic dysentery. It was, however, non-pathogenic to animals.

Bernheim[40] reported that wholly avirulent Klebs-Löffler bacilli became virulent if cultivated on streptococcus filtrates, while the Hofmann-Wellenhof bacillus did not.

Bardach[41] raised the virulence of slightly pathogenic diphtheria bacilli by passage through the dog.

Trumpp[42] converted an avirulent *B. pseudodiphthericus* (it produced acid, however) into a typically virulent form by passage through guinea-pigs simultaneously with sub-lethal doses of diphtheria toxin.

Spronck[43] observed that *B. pseudodiphthericus* and *B. xerosis* produced slight local lesions, such as induration and edema, at the point of inoculation which were similar to those produced by true diphtheria bacilli of low virulence.

Hewlett and Knight[44] changed a typically virulent *B. diphtheriae* into a non-virulent organism of the pseudodiphtheria type by 17 hours exposure to a temperature of 54° C. Subsequent attempts to repeat this experiment were unsuccessful. They also report changing a *B. pseudodiphthericus* strain into a form typically virulent by cultivation and by passage through guinea-pigs.

Shattock[45] investigated the possibility of increasing the virulence of *B. diphtheriae* by means of sewer air. He found that two months cultivation of a slightly virulent Klebs-Löffler bacillus in a current of sewer air did not exalt its pathogenicity.

Martin[46] raised the virulence of feebly pathogenic diphtheria bacilli by cultivation in collodion sacs in the peritoneal cavity of rabbits.

Bergey[47] was unable to give virulence to avirulent pseudodiphtheria forms.

Davis[48] described 12 diphtheroid strains, isolated from cases of scarlatinal otitis media, which were pathogenic to guinea-pigs on subcutaneous inoculation, producing peritonitis and septicemia. Antitoxin afforded no protection against any of them. Williams[49] simultaneously reported finding the same organism.

Richmond and Salter[50] and Salter[51] changed five pseudodiphtheria bacilli into forms resembling the typical *B. diphtheriae* and specifically virulent for guinea-pigs, by passage through gold-finches. The organism now formed acid in broth and its action was neutralized by diphtheria antitoxin.

De Simoni[52] reported success in making pseudodiphtheria bacilli virulent by growing them on the surface of old tetanus cultures.

Levy and Fickler[8] described a diphtheroid which they have named *Corynebacterium lymphae vaccinalis.* While producing no soluble toxin, it causes abscess and chronic suppuration in animals.

Neumann[53] obtained an "hereditary" attenuation of the Klebs-Löffler bacillus by the prolonged action of increased atmospheric pressure.

Martin,[46] Spronck,[54] Park and Williams,[55] Nicolle,[56] and Nicolas and Arloing[57] have exalted the virulence of this species by means of special cultural media to favor stronger toxin production.

Levy[58] repeated Roux and Yersin's work in increasing virulence by animal passage in symbiosis with streptococci. He was unable to make pseudodiphtheria strains virulent.

Lesieur[20] found that of 30 strains of pseudodiphtheria bacilli, 13 produced appreciable quantities of toxin, and 10 more formed traces of it. Of 40 strains of Klebs-Löffler bacilli, 12 produced toxin in only minute quantities and three produced none whatever. He succeeded in causing diphtheria in guinea-pigs with Hofmann-Wellenhof bacilli both by inoculation with large quantities of unchanged bacilli and by raising the virulence of the latter by collodion sac animal passage. The morphology changed at the same time as the virulence. Virulent Klebs-Löffler forms were made to lose their pathogenicity by long exposure to diffuse light. This has also been accompanied, as noted above, by a transformation to the pseudodiphtheria morphology. Further, he was able to make two out of four strains of *B. pseudodiphthericus* virulent by repeated transfers in broth and one out of three by a single passage through broth in symbiosis with *Staph. pyogenes aureus.*

Ohlmacher[21] found strains of *B. pseudodiphthericus* which were virulent to guinea-pigs. He also exalted the virulence of bacilli of the short, solid-staining type by passage through a guinea-pig.

Cobbett[32] investigated the organisms secured in an epidemic at Cambridge. He was able to find no intermediate degrees of virulence among the Klebs-Löffler bacilli and none of the Hofmann-Wellenhof type were virulent.

Williams[59] denies that Klebs-Löffler bacilli in the throats of diphtheria patients change to the pseudodiphtheria type, and states that they do not alter when transferred to other throats. Moreover, only a slight decrease in virulence results from prolonged cultivation of *B. diphtheriae* on artificial media. The experiments of increasing the virulence in non-pathogenic strains by symbiosis with streptococci and by passage through gold-finches were repeated but could not be confirmed.

Ruediger[60] described pseudodiphtheria bacilli obtained from scarlatinal throats which were virulent to guinea-pigs.

Graham-Smith[61] found an organism resembling that described by Davis, in a diphtheria epidemic.

Hamilton[23] collected some 15 diphtheria strains pathogenic to guinea-pigs but which did not produce the lesions of the Klebs-Löffler bacillus in them, nor produce soluble toxin. Antitoxin did not protect guinea-pigs against them.

Graham-Smith[34] obtained results similar to those of Cobbett, that is, he found no partially attenuated Klebs-Löffler, nor any virulent Hofmann-Wellenhof bacilli.

Zinsser[62] studying some 79 diphtheria strains found no virulent pseudodiphtheria or xerosis bacilli, but two diphtheroids had some slight pathogenicity, and one of them gave some immunity against *B. diphtheriae.*

Immune serum reactions.—De Martini[63] reported that pseudodiphtheria bacilli grow freely in antitoxin while *B. diphtheriae,* whether virulent or avirulent, does not develop. This interesting observation has never been confirmed.

Spronck,[43] in 1896, described a method, which is now known as "Spronck's Test," for distinguishing between the Klebs-Löffler bacillus and *B. pseudodiphthericus* and *B. xerosis.* He injected guinea-pigs with diphtheria antitoxin and then inoculated them with the culture to be examined. If the latter happened to be true diphtheria bacilli the animal showed complete protection, if not, local infiltration and edema was produced as in the controls. Of course, the test is not applicable to wholly avirulent strains. Spronck's results were soon confirmed by several investigators including C. Fränkel[64] who was so impressed with their significance that he was lead to retract his acceptance of Roux and Yersin's theory of the biologic identity of the members of the diphtheria group.

Glücksmann[65] showed that animals immunized with *B. pseudodiphthericus* are not protected against *B. diphtheriae.*

Bergey[46] could produce no immunity against virulent by means of non-virulent pseudodiphtheria bacilli.

Salter[51] reported that he had found in filtrates of *B. pseudodiphthericus* substances identical with the toxoid produced by the Klebs-Löffler bacillus.

Nicolas[66] discovered that antidiphtheritic serum agglutinated the Klebs-Löffler bacillus, thus offering a simple and excellent means of diagnosis. On repeating the experiments, however, Nicolle[67] was unable to confirm his results. Nicolas,[68] on going more deeply into the matter, found that the agglutinability of *B. diphtheriae* is not constant and that the differences which had been observed were due to differences in the races of bacilli used. He stated that no relation between virulence and agglutinability appeared to exist. His results are confirmed in general by Bruno.[69]

Lubowski[70] also employed agglutination experiments to separate the true from the false diphtheria bacillus. He immunized goats with avirulent Klebs-Löffler bacilli and secured a serum which agglutinated 23 races of *B. diphtheriae* but had no action on *B. pseudodiphthericus.* On the other hand, Lesieur[71] found there was no difference in behavior toward a specific serum *in vitro* between the true diphtheria and the pseudodiphtheria bacilli. He also states that *B. pseudodiphthericus* produces a body similar to, if not identical with, the toxone of *B. diphtheriae.*

Gordon[33] found that there were great variations in the results of agglutination experiments even with Klebs-Löffler strains, thus confirming the observations of Nicolas. He thinks this depends on whether or not the bacilli used as antigens were recently isolated.

Schwoner[72] thinks reliable results are obtainable in differentiating *B. diphtheriae* from *B. pseudodiphthericus* by means of agglutination in specific sera.

Lambotte[73] found evidence that the amboceptors of *B. diphtheriae* and *B. pseudodiphthericus* are not identical.

Cobbett[74] and likewise Hewlett[75] were unable to confirm the results of Salter in regard to toxoid production. The same conclusion was reached by Petrie[76] for he found that filtered cultures of pseudodiphtheria bacilli do not immunize horses so that diphtheria antitoxin is produced.

Hamilton and Horton[77] reported that the antibodies formed on immunization of goats and rabbits with virulent pseudodiphtheria bacilli are distinct from those produced by avirulent pseudodiphtheria forms as well as those of *B. diphtheriae.*

Cultural characters.—Abbott[78] found an organism indistinguishable from the nonpathogenic *B. diphtheriae* except in that it formed a "dirty brown layer" on potato.

McClure[79] and also Gordon[25] have described diphtheria-like bacilli which strongly acidified and coagulated milk and Klein[5] isolated a similar organism, *B. diphtheroides,* which not only strongly acidified and coagulated milk, but also liquefied Löffler's inspissated serum and stained with difficulty with the ordinary anilin dyes.

Zupnik[39] found two distinct strains existing symbiotically in the same culture of *B. diphtheriae.* They differed in regard to appearance in broth, vigor of growth, the morphology of the colonies, Gram stain, and pathogenicity.

Graham-Smith[34] uses as one means of differentiating between the Klebs-Löffler and the Hofmann-Wellenhof types the character of their growth on his potato-agar medium. He described a number of diphtheroids secured from different parts of the body some of which actually coagulate milk, produce indol, reduce nitrates, liquefy Löffler's serum and gelatin, and form a yellow growth on potato.

Schwoner[80] showed that about 70 per cent of the true diphtheria strains are able to cause hemolysis in rabbits' corpuscles (this has been observed by others also), while *B. pseudodiphthericus* (typical) failed to produce hemolysis.

Löffler's serum and agar.—Regarding the growth of the members of the group on these media, it is unnecessary to take time for a rehearsal of the literature, as everyone who has worked with diphtheria bacilli knows how extremely variable they are in this respect.

Acid fermentation of carbohydrates (and alcohols).—To facilitate comparison, as well as to save time, I have tabulated the results of the important investigations on this point. In most cases 1 per cent of the sugar was used, usually dissolved in broth, but in some cases in the serum-water medium of Hiss[81]. In all cases the production of acid was recognized by means of indicators in the medium.

TABLE 1.

Author	Dextrose	Levulose	Galactose	Maltose	Saccharose	Dextrin	Lactose	Mannite	Glycerin	Arabinose	Raffinose	Dulcite	Glycogen	Erythrite	Starch	
T. Smith[82]	+				—		—									*B. diphtheriae*
Blumenthal[83]	+						+									
Axenfeld[84]	+															
Franke[85]	+															
Martin[46]	+	+	+	—	+		—	—	+	—	—	—	—	—	—	
Bronstein and Grünblatt[86]	+															
Knapp[87]	+			+	—	+	—	+								
Hamilton and Horton[77]						+										
Graham-Smith[88]	+	+	+	+	—	+	±	—	+							
Rothe[89]	+	+		±	—		—	—								
Zinsser[62]	+	+	+	+	—	+	—	—				—				
Arkwright[90]	+	+	+	+	—		+	—								
Eyre[91]	*x*						*x*									*B. pseudodiphthericus*
Peters[92]							+									
Axenfeld[84]	*x*															
Franke[85]	—															
Lawson[93]	—															
Gordon[33]	—															
Bronstein and Grünblatt[86]	—															
Denny[22]	*x*															
Knapp[87]	—			—	—	—	—	—								
Hamilton and Horton[77]						—										
Graham-Smith[88]	—	—	—	—	—	—	—	—	—							
Benham[94]	—			—	—		—									
Rothe[89]	—	±		—	?		—	—								
Zinsser[62]	—	—	—	—	—	—	—	—				—				
Arkwright[90]	—	—	—	—	—		—	—								
Eyre[91]	—															*B. xerosis*
Axenfeld[84]	—															
Franke[85]	*x*															
Denny[22]	+															
Knapp[87]	+			+	+	—	—	+								
Graham-Smith[88]	+	+	—	—	+ ?	—	—	—	+							
Benham[94]	+			—	—		—									
Rothe[89]	—	—		—	—		—	—								
Zinsser[62]	+	+	+	+	+	—	—	—				—				
Gordon[33]	—															
Gordon[33]	+															
Graham-Smith[88]	+	+	+	—	—	—	—	—	—		*B.*	*cor*	*yzae*	*seg*	*ment*	*osus*
Benham[94]	+			+	+		+									
Arkwright[90]	+	+	—	—	—		—	—								
Graham-Smith[88]	±	±	±	±	—	—	—	±	—							
Zinsser[62]	+	+	+	+	—	±	—	—				—		*Dip*	*hthe*	*roids*
Arkwright[90]	+															

NOTE.—Kurth[15] has described a *B. pseudodiphthericus acidum faciens* which forms as much acid in dextrose as the Klebs-Löffler bacillus.

+ = acid production. — = alkali production. *x* = no change.

The foregoing review requires no discussion to convince one that aside from the immunity and fermentation reactions the means of differentiating accurately between the members of this group are valueless. As for the serum reactions, however, suffice it to say that as yet it is unknown whether specific antigenic capacity is a species or merely a racial character, indeed, even the work on this subject in connection with the diphtheria group, as cited above, contains much that strongly hints at the latter interpretation. Until this is disproved, then, evidence that the diphtheria group comprises more than one species, apparently must be deduced from fermentative characters alone. A glance at the preceding tabulation shows that there are many discrepancies in the results reported, but more careful perusal almost satisfies one that certain of the reactions stand out as being comparatively undisputed. Compiling these presumably valid reactions, it is found that they form a scheme whereby the three important members of the group might be sharply differentiated, thus:

TABLE 2.

	Dextrose	Dextrin	Saccharose
B. diphtheriae............	+	+	—
B. pseudodiphthericus.	—	—	—
B. xerosis................	+	—	+

Now, it is quite readily conceivable that these alone may be the essential diagnostic species characters, and in that case the lack of uniformity in behavior toward the other sugars is of no consequence. Inasmuch, however, as the propriety of dividing the diphtheria group into distinct species appears to rest exclusively upon these few reactions, it seems highly important to settle their validity as species characters. To justify their use as species characters these fermentative powers must fulfil two requirements. In the first place, they must be permanent and hence unalterable in spite of prolonged artificial or natural selection so that organisms of the species from whatsoever source will have practically the same zymogenic characters. In the second place, there must be no intermediary forms, for this would indicate either that members of the species could gradually lose these characters or else that the latter could be acquired by another species. If, therefore, one or both of these conditions can be demonstrated as lacking in this case, we must relinquish the fermentation test.

The following study was undertaken for that purpose, and also, incidentally, to determine how far such a fundamental biologic character as enzyme production can be altered artificially.

The problem was approached in two ways. The first was to secure as many strains of this group as possible and determine by titration the exact amount of acid produced in the different sugars, with the object of learning to how great a degree the fermentative powers of these bacteria vary in nature. If it were to transpire that the organism could be grouped into two or more definite aggregations on the basis of the extent of their zymogenic power, the presumption would be that this was constant, while, on the other hand, if it were found that such grouping was not possible because of the occurrence of intermediate or transitional forms, this would necessarily have to be regarded as evidence of the instability of the characters and of the likelihood that the latter can be assumed by other types in the group.

For this purpose 103 strains of the diphtheria group were secured, some from the laboratories of other universities, but most were isolated from smears on Löffler's medium sent to the Chicago City Health Department for diagnosis.* Immediately after isolation the organisms were inoculated into tubes containing 1 per cent of dextrose, dextrin, maltose, or saccharose in sugar-free infusion broth, incubated at 37° C. for three days, and the amount of acidity produced was determined by titration with N/20 NaOH. The object of titrating for the exact amount of acid instead of merely demonstrating the presence of acid by adding an indicator to the medium as others have done, was that in this way can be detected the existence of intermediate forms, the crucial point in the whole question, while by the older method this is impossible. By the latter can be learned whether a given bacterium does or does not produce acid but no information is given regarding the extent to which acid is produced. This can be obtained only by means of titration. In other words, the indicator method emphasizes uniformity and obscures the variations, while the titration method emphasizes the variations.

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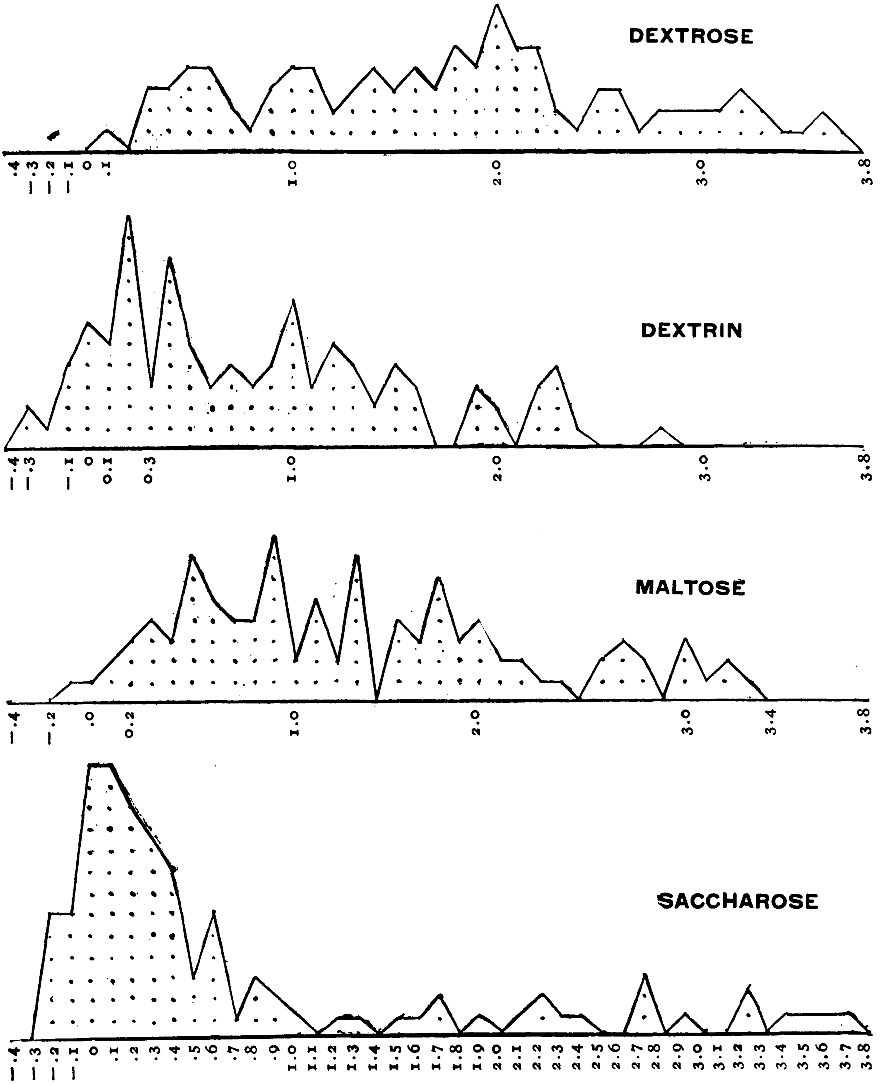
* I am indebted to Dr. Weber of the Health Department Laboratory for the smears and I take this opportunity to thank him.

TABLE 3.

No.	Strain	Dex-trose	Dex-trin	Mal-tose	Sac'a-rose	No.	Strain	Dex-trose	Dex-trin	Mal-tose	Sac'a-rose
1	15–	4.0	2.3	3.1	2.1	53	129	1.8	1.5	1.3	0.0
2	Herdman	3.7	0.5	0.6	3.7	54	410	1.8	2.3	2.7	2.2
3	683C	3.6	1.2	3.0	3.2	55	Buchanan–	1.8	0.4	0.7	0.2
4	Hudson	3.6	−0.2	1.0	3.5	56	Minn. D	1.7	1.2	0.9	0.3
5	72	3.5	1.2	0.9	2.4	57	155	1.7	0.9	2.2	0.8
6	477–	3.4	1.6	3.3	2.3	58	874	1.7	2.4	1.8	0.4
7	308–	3.3	1.5	1.3	0.0	59	58A	1.6	1.0	1.1	0.3
8	329–	3.3	2.2	1.4	−0.2	60	167	1.6	1.0	1.8	0.6
9	114	3.2	0.25	2.8	−0.2	61	594	1.6	1.0	1.7	0.1
10	Granger	3.2	0.4	0.5	3.6	62	715	1.6	0.7	0.8	0.5
11	65	3.2	−0.3	0.4	3.4	63	338	1.5	0.7	1.7	0.35
12	277	3.1	−0.3	0.5	3.2	64	682	1.5	0.5	1.3	1.7
13	442–	3.1	0.3	0.9	2.9	65	Bapt–	1.5	0.1	1.9	0.0
14	19–	3.0	0.4	3.0	0.6	66	27A	1.4	0.1	1.6	1.5
15	148–	3.0	1.9	3.2	2.7	67	74–	1.4	0.9	0.8	2.1
16	85	2.9	2.0	2.4	0.4	68	138	1.4	1.0	1.8	0.1
17	633	2.9	2.3	3.2	0.0	69	776A	1.04	0.0	1.3	0.0
18	23D–	2.8	0.5	1.8	2.7	70	M. I.–	1.3	0.9	1.1	0.9
19	520	2.8	1.3	2.7	0.0	71	191	1.3	1.1	1.2	0.4
20	342	2.7	1.4	2.0	0.6	72	702	1.3	0.5	0.6	0.5
21	8–	2.6	1.0	1.6	0.2	73	32	1.2	0.2	0.9	1.0
22	Davis–	2.6	0.5	1.3	0.2	74	572	1.2	2.2	1.0	−0.1
23	491A	2.6	1.5	2.7	0.3	75	13–	1.1	0.9	2.2	0.1
24	23E–	2.5	0.2	1.4	2.7	76	388	1.1	0.1	0.8	1.6
25	370	2.5	1.9	2.3	0.2	77	441–	1.1	0.7	1.1	0.4
26	528	2.5		0.1	1.9	78	K	1.1	0.0	1.3	0.8
27	739	2.4	1.5	2.0	0.2	79	Park–	1.0	0.4	0.6	0.05
28	20B	2.3	1.3	1.3	0.9	80	252	1.0	0.7	1.1	0.3
29	214–	2.3	1.1	2.0	0.4	81	484–	1.0	0.4	0.7	0.4
30	70–	2.2	1.35	3.0	0.0	82	Brown P. V–	1.0	0.2	0.8	0.2
31	4–	2.2	0.8	2.1	0.8	83	222	0.9	0.4	1.2	0.3
32	46	2.2	0.4	2.1	−0.05	84	440–	0.9	0.6	0.9	0.6
33	134	2.2	1.9	1.9	0.3	85	W	0.9	0.2	0.2	1.2
34	848	2.2	0.8	2.6	0.1	86	386B	0.8	0.2	0.4	0.1
35	45C	2.1	0.2	0.9	1.7	87	220	0.7	−0.1	0.4	−0.2
36	115A	2.1	2.0	2.8	0.2	88	Calhoun	0.7	0.0	0.0	0.1
37	211	2.1	0.8	−0.1	0.7	89	Minn. P.–	0.6	0.2	0.6	−0.2
38	239A	2.1	1.6		0.4	90	G	0.6	0.0	0.5	0.1
39	551	2.1	2.3	1.7	0.15	91	Disp.	0.6	−0.1	0.5	−0.2
40	Ch. P.	2.0	1.1	0.5	0.6	92	Simpson–	0.6	1.0	0.5	0.1
41	44	2.0	2.8	0.9	0.6	93	Harv. P.–	0.5	0.2	0.6	−0.1
42	Kyes	2.0	1.3	2.2	0.1	94	39	0.5	0.3	0.7	0.0
43	73B	2.0	0.6	1.1	0.5	95	V	0.5	0.2	0.2	0.4
44	284–	2.0	0.0	1.6	−0.1	96	Gage–	0.5	0.4	0.7	0.3
45	548	2.0	2.2	2.6	0.0	97	335	0.4	0.1	0.1	0.15
46	967	2.0	1.6	1.8	1.3	98	659	0.4	−0.1	0.3	0.1
47	78	1.9	0.6	0.9	0.2	99	811	0.4	−0.1	0.3	0.0
48	103–	1.9	1.0	1.4	−0.1	100	89	0.3	0.1	0.5	−0.2
49	516	1.9	1.2	1.8	0.0	101	431–	0.3	0.0		0.1
50	683A	1.9	0.4	0.2	0.0	102	Brown P. VI–	0.3	0.2	0.3	0.0
51	16B	1.8	1.2	1.6	−0.05	103	Brown P. 31–	0.1	0.2	0.3	0.1
52	50	1.8	1.4	1.9	0.2						

The results of the experiment, which are shown in Table 3 arranged in the order of the dextrose-fermenting power of the strains, demonstrate that the forms cannot be separated on the basis of their fermentations into definite groups. This is shown more clearly when these data are plotted in frequency curves. Inspection of these (Chart 1) reveals the fact that in the case of none of the four carbohydrates is it possible to make a sharp line of division at any point. It must be admitted that the results of previous investigations would cause one to expect that the curves, even if continuous, would reveal several "peaks" separated by distinct "valleys" to account for the rather clean-cut results of the indicator method experiments, but a moment's reflection will explain the apparent discrepancy for with an indicator the most level titration "plateau" may be divided into two sharply separated groups.

CHART 1.

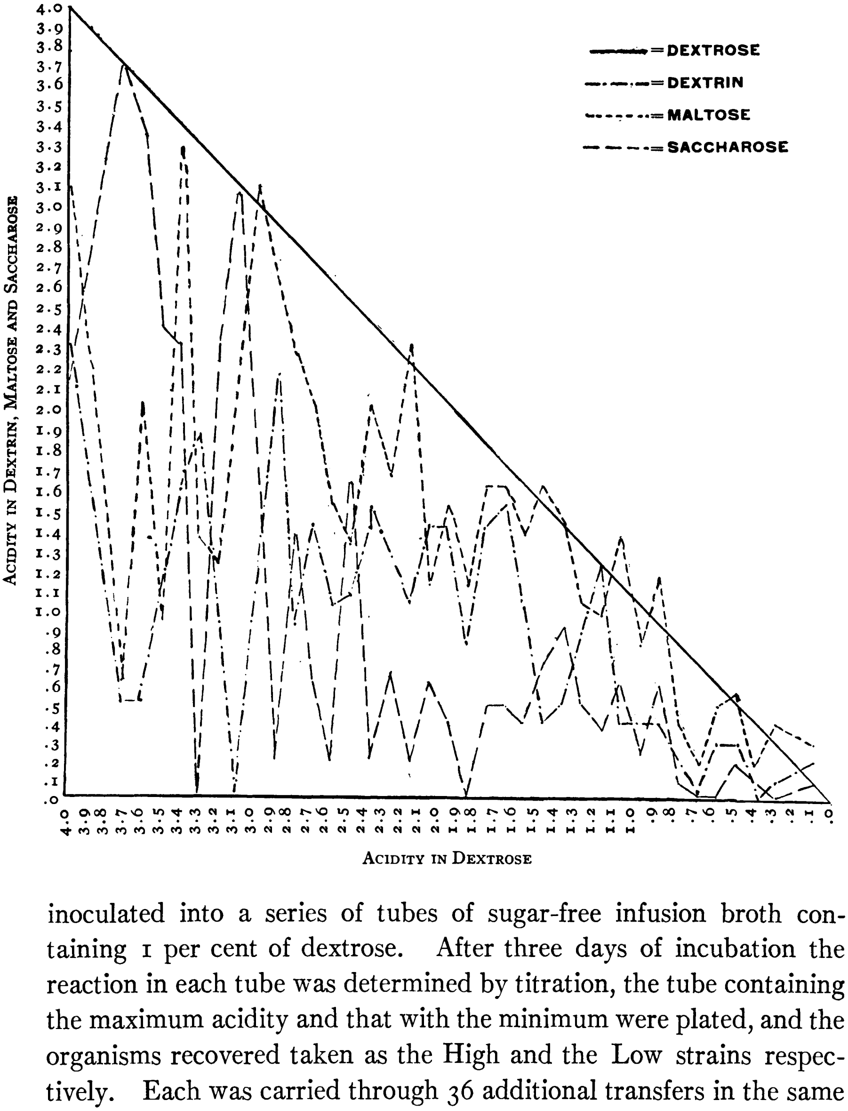


The curves, although of the same general significance, show minor differences among themselves that are of sufficient importance to merit mention. In the first place, the type of each curve is different. The extremes are dextrose and saccharose, the former showing a remarkably evenly graded distribution of zymogenic power over an extremely wide range (almost 4 per cent) while the latter, although really covering an even more extensive range, is for the most part limited to a variation of only about 1 per cent and so situated as to demonstrate that the majority of the members of the diphtheria group possess rather small power to ferment saccharose. The curves of dextrin and maltose are intermediate between the other two, the former resembling saccharose more closely and the latter dextrose. In the second place, there does not appear to be any relation between high fermentative power with one sugar and the ability to break up other carbohydrates, that is, a bacterium, whose fermentative powers in respect to one sugar are exaggerated, may have a subnormal capacity in regard to other sugars and *vice-versa,* although when the fermenting capacity for dextrose is low that for the others is correspondingly depressed. The point is presented graphically in Chart 2 where the dextrin, maltose, and saccharose fermentations are plotted in relation to the dextrose.

The next experience was to determine the limits of the variability in the zymogenic power. Its bearing on the general problem is that if prolonged artificial selection should prove unable to change materially the fermentative character of one of these organisms, strong evidence of the permanence of that character would be afforded. On the other hand, if selection can materially alter the power, exalt or depress it at the will of the experimenter, it is but reasonable to expect similar changes in nature and thus would be explained the occurrence of almost every degree of development of the character in different strains, as shown in Table 3. In fact, such an alteration would be reasonably conclusive proof that the fermentative properties of the members of this group cannot be utilized as a differentiating species character. Such actually proved to be the case in this experiment.

A culture of the Klebs-Löffler bacillus, isolated from a clinical case by Professor Preston Kyes of the Pathology Department, was plated, one colony isolated (the descendant of a single cell presumably), and

CHART 2.



inoculated into a series of tubes of sugar-free infusion broth containing 1 per cent of dextrose. After three days of incubation the reaction in each tube was determined by titration, the tube containing the maximum acidity and that with the minimum were plated, and the organisms recovered taken as the High and the Low strains respectively. Each was carried through 36 additional transfers in the same manner, that with the maximum acidity being the only organism saved and carried on in the High strain, and similarly that with the minimum acidity in the Low series. The tubes were incubated for from two to four days before titration, according to convenience, but lack of constancy in this particular cannot be a factor in the results obtained since the corresponding High and Low strains were always run through together, and it is not the actual amount of acidity produced that is the important element in this case but the difference between that formed by the maximum of one and the minimum of the other strain. To guard against contamination, the strains were usually plated weekly. It was not felt necessary to do this after each transfer for certainly it does not hasten the process of adaptation, on the contrary, if one happens to pick out a colony belonging to the minority of the less adaptable bacilli, results are retarded, at least in the High strains. It seems reasonable to hold that the most adaptable variant in each culture will multiply most rapidly so that when inoculated from tube to tube by means of the platinum loop an excess of this variant is carried over to begin with and more rapid results will be obtained than by inoculation with a colony possibly consisting of the least adaptable variant in the culture. As a matter of fact, indeed, it was not infrequently noticed in the Low series that the culture following plating gave an appreciably smaller amount of acid than did those immediately preceding with which direct inoculation was practiced. Another factor, of less importance, is that direct inoculation saves at least 24 hours in time on each series.

The results of the titrations* in this experiment are shown in Table 4, and the curves of the acid production by the maximum variant in the High and the minimum variant in the Low strains of

TABLE 4.

The **bold-face** figures are those of the cultures carried over for the inoculation of the following series. Roman numerals indicate series. H = High strain, L = Low strain.

ORIGINAL	I		II		III		IV		V		VI		VII		VIII	
	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L
2.3	2.1	2.0	1.8	1.9	1.9	1.7	1.0	1.6	2.5	1.6	1.9	1.9	2.3	1.6	2.2	**1.7**
2.2	2.2	1.5	1.6	2.0	1.7	1.6	1.6	1.8	2.4	2.1	1.9	1.8	2.0	2.0	2.0	1.9
2.2	**2.4**	1.9	1.9	1.7	1.9	1.6	1.5	1.8	**2.6**	2.0	1.8	1.9	2.3	2.2	2.4	1.9
2.0	1.7	1.7	**2.0**	2.0	2.0	1.5	1.5	1.6	2.4	1.8	2.0	2.1	**2.4**	2.2	**2.5**	2.3
2.1	1.6	2.0	1.7	1.8	2.0	1.7	1.3	1.7	2.1	1.8	2.2	2.0	1.9	1.6	2.3	2.3
2.1	1.7	**1.7**	1.6	1.7	1.8	1.6	1.4	1.9	2.1	**1.4**	2.0	2.0	2.2	1.7	2.3	2.0
2.3	1.6	2.3	1.9	1.7	2.0	1.5	1.3	1.5	2.3	1.7	2.1	1.9	2.2	1.6	2.3	1.8
1.9	1.7	2.1	1.5	1.9	1.5	1.6	1.3	**1.5**	2.3	2.1	1.9	2.1	2.0	2.2	2.1	1.8
1.9	1.9	1.7	1.6	2.1	**2.1**	1.6	1.4	1.9	2.2	1.7	**2.2**	1.8	2.0	2.0	2.2	2.1
2.3	1.9	2.1	1.6	1.8	2.1	1.7	1.4	1.6	2.0	1.7	2.0	2.1	2.3	**1.4**	2.1	1.8
2.0	1.9	1.7	1.8	1.8	2.0	**1.5**	1.5	2.0	2.4	2.1	1.9	1.9	2.1	1.8	2.4	2.0
1.9	2.0	1.9	1.6	**1.6**	2.1	1.5	1.4	1.6	2.1	1.6	1.8	1.9	2.0	1.7	2.4	1.7
1.9	1.7	1.7	1.8	1.9	1.8	1.6	**1.7**	1.7	2.1	1.6	2.1	2.0	1.9	1.6	2.2	2.0
2.0	1.6	1.9	1.8	1.9	2.0	1.5	1.0	1.5	2.1	1.8	2.1	**1.8**	1.9	1.5	2.0	1.8
2.1	2.0	1.7	1.8	2.0	2.0	1.8	1.4	1.8	2.4	2.1	2.0	2.0	2.2	1.6	2.3	2.1

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* The titrations were performed for me by Miss Ethel Lowenthal, a student in the laboratory.

TABLE 4.—*Continued.*

IX		X		XI		XII		XIII		XIV		XV	
H	L	H	L	H	L	H	L	H	L	H	L	H	L
2.8	1.7	**3.1**	2.1	3.3	2.4	2.9	2.3	3.1	2.0	2 4	2.0	3.2	**1.5**
2.7	2.4	2.9	2.2	3.3	2.2	2.6	2.5	2.7	2.6	2.2	2.6	3.0	2.3
3.1	1.2	2.5	2.6	3.0	2.5	2.7	2.5	2.7	2.0	2.7	2.0	3.3	2.3
3.0	1.9	3.1	2.4	2.6	2.8	**3.4**	1.9	2.5	2.0	**3.1**	2.0	2.7	2.1
2.9	1.9	2.7	2.0	3.1	2.3	3.1	2.3	3.0	1.5	2.7	1.5	2.8	2.2
3.2	2.2	2.8	1.9	**3.4**	2.8	3.2	2.7	2.9	2.3	3.0	2.3	**3.4**	1.7
3.0	1.8	3.0	2.1	2.6	2.3	3.4	**1.8**	2.7	2.2	2.9	**1.4**	3.0	2.0
2.7	**1.2**	3.0	2.4	2.9	2.4	2.6	2.0	2.4	1.7	2.8	1.7	3.0	1.6
3.1	1.9	2.7	**1.6**	3.3	2.6	2.7	2.3	2.8	2.0	3.0	2.0	2.9	1.6
3.1	1.4	2.9	2.3	3.3	2.7	2.5	2.2	2.8	2.5	2.6	1.5	2.5	1.8
2.9	1.4	3.0	2.3	2.5	**2.2**	3.0	2.5	3.0	1.9	2.9	1.9	3.0	2.1
2.8	1.5	2.8	1.9	2.9	2.4	3.1	2.1	2.6	**1.4**	2.7	1.8	3.1	1.6
3.0	1.9	2.8	2.1	3.2	2.5	2.8	2.1	2.9	1.8				
3.0	1.7	2.6	2.1	3.0	2.7	3.0	2.4	**3.2**	2.0				
2.9	1.6	2.6	2.2	3.1	2.3	2.7	2.4	3.0	2.1				

XVI		XVII		XVIII		XIX		XX		XXI		XXII	
H	L	H	L	H	L	H	L	H	L	H	L	H	L
3.1	1.7	2.8	1.1	2.9	2.1	3.5	0.7	4.2	0.8	4.2	0.8	3.0	0.1
2.4	**1.4**	2.5	1.2	2.8	1.4	3.6	**0.2**	4.4	0.8	3.7	0.6	4.1	−0.1
3.1	1.7	3.1	0.9	3.1	1.6	3.0	0.7	4.1	**0.3**	3.3	0.7	4.1	−0.1
2.8	2.1	**3.2**	1.4	**3.3**	2.0	3.3	0.7	4.1	0.8	4.1	1.1	4.0	−0.1
2.8	2.0	3.0	1.8	3.0	2.2	3.3	1.0	4.5	0.7	3.7	**0.6**	3.9	0.0
3.0	1.5	2.7	1.7	3.0	1.6	3.6	0.9	4.0	0.7	3.0	0.7	4.0	−0.1
2.9	1.4	2.9	1.2	2.7	1.7	3.3	0.7	3.8	0.8	3.6	0.8	4.1	−0.2
3.0	1.8	3.0	1.2	2.8	1.4	3.4	0.8	4.1	1.0	3.8	1.0	4.0	−0.1
3.0	1.6	2.9	**0.6**	2.5	2.1	**3.9**	0.6	4.5	0.7	**4.2**	0.7	4.1	0.01
3.2	1.8	2.7	1.6	3.1	1.8	3.4	0.6	4.5	0.5	3.4	0.9	**4.1**	**−0.2**
3.2	1.4	3.1	0.9	3.1	1.9	3.8	1.2	**4.8**	0.8	3.9	0.6	3.4	0.1
3.1	1.9	2.9	0.9	2.6	**1.0**	3.2	0.8	4.0	0.7	3.6	0.7	3.2	0.1

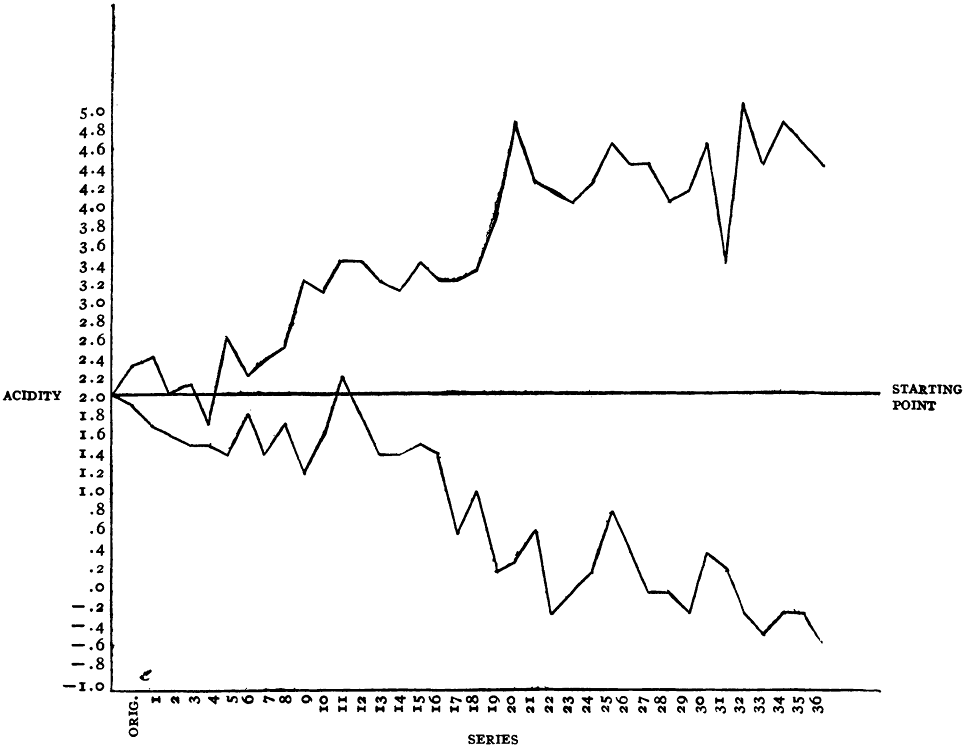
XXIII		XXIV		XXV		XXVI		XXVII		XXVIII		XXIX	
H	L	H	L	H	L	H	L	H	L	H	L	H	L
3.3	**0.0**	3.5	0.6	4.2	0.8	3.9	0.9	3.8	0.6	3.9	0.5	3.3	0.6
3.8	0.4	2.7	0.9	3.2	**0.8**	4.3	0.9	2.9	0.7	3.4	**0.0**	3.6	0.6
3.4	0.1	2.5	0.4	3.9	1.0	3.7	1.2	2.9	0.7	3.8	0.6	4.0	0.2
3.8	0.3	2.6	1.0	**4.6**	1.6	3.5	0.7	3.7	0.9	3.5	0.3	3.2	−0.1
3.9	0.6	2.8	0.2	4.3	1.0	4.0	0.7	3.6	0.3	3.9	0.3	3.9	0.2
3.2	0.4	**4.2**	0.3	4.3	1.2	4.0	**0.4**	4.1	0.7	3.9	0.9	3.9	−0.1
3.9	0.3	2.9	**0.2**	4.0	1.2	**4.4**	0.9	3.4	0.7	**4.0**	0.5	3.8	0.4
3.0	0.6	3.1	0.2	3.5	1.0	4.1	1.1	3.8	1.0	3.6	0.9	3.4	0.1
3.9	0.2	3.1	0.6	3.9	1.3	3.7	1.4	3.6	0.5	3.8	1.1	**4.1**	0.3
4.0	0.3	3.5	0.3	4.1	1.6	3.9	1.1	3.2	**0.0**	3.6	1.1	3.7	0.3
4.0	0.3	3.9	0.2	3.7	1.1	3.9	0.8	4.0	0.7	3.0	0.6	3.6	**−0.2**
3.6	0.4	3.8	0.3	4.1	1.2	3.9	1.2	**4.4**	0.6	3.1	0.8	3.8	0.0

XXX		XXXI		XXXII		XXXIII		XXXIV		XXXV		XXXVI	
H	L	H	L	H	L	H	L	H	L	H	L	H	L
3.7	1.2	**3.4**	0.5	4.6	0.2	4.0	0.3	**4.8**	0.1	3.9	0.5	4.0	0.2
4.1	1.0	2.6	0.3	4.4	0.0	**4.4**	−0.1	4.0	0.7	3.9	0.1	4.1	0.3
4.6	0.6	2.2	1.1	4.6	0.5	4.1	0.2	4.2	0.4	3.8	0.1	3.5	−0.2
4.4	0.7	2.8	1.2	4.3	0.6	4.0	0.4	3.7	0.0	**4.6**	0.2	3.6	0.0
4.6	0.7	2.9	0.7	**5.0**	0.1	4.2	−0.2	4.4	0.0	3.9	0.4	3.2	0.1
4.0	**0.4**	2.8	1.0	4.9	−0.1	3.7	−0.1	4.4	**−0.2**	4.2	0.0	3.9	0.6
3.8	1.3	2.5	0.5	4.1	0.3	3.3	0.1	4.5	−0.2	4.4	−0.1	3.6	−0.1
3.1	0.8	3.1	0.9	4.4	0.0	4.1	0.2	4.4	0.3	4.0	0.1	**4.4**	−0.3
4.1	0.5	3.0	0.9	4.7	**−0.2**	4.4	0.0	4.2	0.1	3.7	0.3	3.9	0.0
4.0	1.1	2.8	1.2	4.6	0.0	3.5	**−0.4**	4.1	0.0	4.1	**−0.2**	4.1	−0.1
3.9	1.5	3.2	0.8	4.0	0.1	3.8	−0.1	3.9	0.1	4.0	0.0	3.8	**−0 5**
4.3	0.6	3.0	**0.2**	4.1	0.5	3.7	−0.1	4.5	−0.1	3.8	0.3	3.9	−0.1

each series are plotted in Chart 3. Although discouragingly close for the first few weeks the curves then begin to diverge very markedly, and by the 20th series the difference between the two is most striking. The extent of the variation effected in the two strains is more easily appreciated by a glance at Chart 4 where the difference in acid-forming power is plotted. The steady and comparatively rapid development of this variation is highly significant, showing how unstable this property is, and how misleading it might be to utilize it as the criterion by which to determine whether a given organism is merely a harmless saprophyte or belongs to one of the most dangerous of bacterial species. As marked as the difference between any diphtheria and pseudo-diphtheria strains in this particular is that between the High and the Low strains at the conclusion of the experiment, for while the former is a rapid and vigorously fermenting organism, producing an extremely

CHART 3.

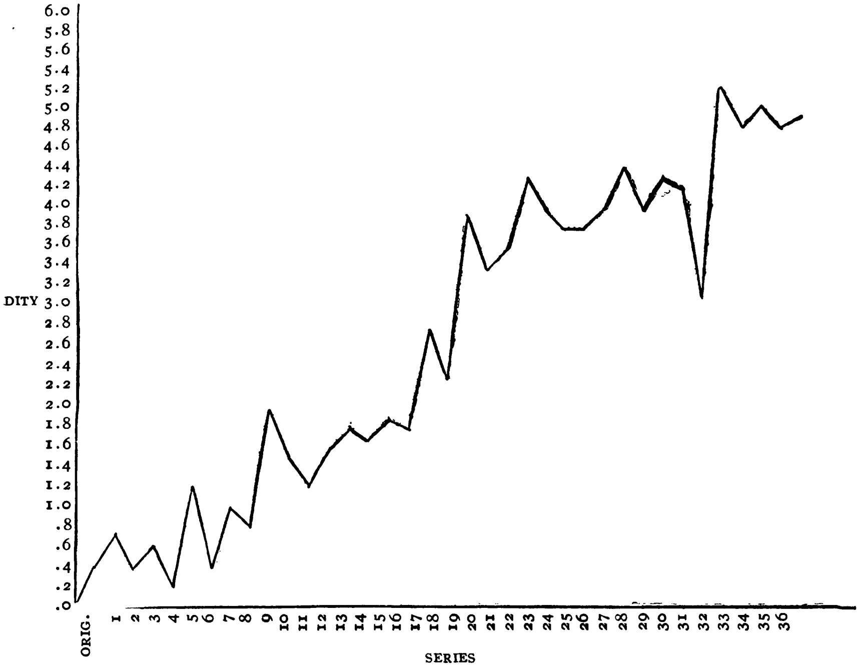
ACID PRODUCTION IN MAXIMUM OF HIGH AND MINIMUM OF LOW STRAINS



large amount of acid in dextrose broth, the latter not merely forms no acid but the medium actually becomes alkaline (as a matter of fact this was not observed, but that is inconsequential for the reaction of the medium was made acid to begin with and the acidity was correspondingly diminished in this case). Would one have hesitated to declare the High strain *B. diphtheriae* and the Low strain *B. pseudodiphthericus*, assuming both to be avirulent to guinea-pigs, and their history unknown?

CHART 4.

VARIATION IN ACID PRODUCTION BETWEEN MAXIMUM OF HIGH AND MINIMUM OF LOW STRAINS IN EACH SERIES



In this connection it was of interest to learn what other changes, if any, accompanied this variation in the dextrose fermenting power. The morphology of the two strains was compared with the original parent strain after four transfers on Löffler's inspissated serum. By Neisser's stain, the parent was a slender granular form as it had been originally, and the two daughter strains were likewise granular though rather shorter than the mother, particularly the High strain, but the difference was by no means marked. The High strain grew appreciably, though not pronouncedly, more vigorously than the Low on Löffler's serum during the first 24 hours and its surface seemed to be somewhat more moist. On agar no differences could be detected between the strains, both growing better than the parent. Their behavior toward the other test carbohydrates is shown in the subjoined table.

TABLE 5.

Strain	Dextrose	Dextrin	Maltose	Saccharose
Parent.............	2.0	1.3	2.2	0.1
High..............	4.4	1.1	1.2	0.6
Low..............	−0.5	1.1	0.3	0.2

It is difficult to understand these changes in the zymogenic property for other carbohydrates, but they nevertheless emphasize the point already made in regard to the instability of this character.

Regarding pathogenicity, the results, unfortunately, were not very satisfying. Twenty-four-hour broth cultures of the Parent, High, and Low strains were inoculated subcutaneously into test-weight guinea-pigs in doses of 0.5 c.c. each, while 200 units (1 c.c.) of antitoxin was simultaneously given intra-peritoneally. The animal receiving the High strain died in two and one-half days with postmortem lesions typical of diphtheria. The other two animals were quite unaffected except for a little local infiltration at the site of inoculation. It should be mentioned that the Parent strain had originally been extremely virulent for guinea-pigs. As yet I have not repeated the experiment with small quantities of antitoxin and with none at all. Even so, however, the variability of the character is demonstrated.

We may summarize as follows: Separation of the members of the diphtheria group into two or more species on the basis of morphology, staining properties, character of growth, or pathogenicity is not justifiable because of inconstancy. The same applies to immunity reactions, which have the additional objection that their significance as species reactions is as yet unknown. The only character which experience has not shown to be untrustworthy is that of difference in behavior toward carbohydrates. It is unnecessary to discuss here the propriety of establishing species on the basis of but a single differential character, but the foregoing experiments demonstrate that even these fermentation properties are not species characters since (*a*) almost every intermediate grade of development can be found by proper methods in different members of the group as they exist in nature, and (*b*) the extent to which the zymogenic power is exhibited can be readily and markedly altered at will by artificial selection. On the strength of these facts, then, I venture to suggest that the division of the diphtheria group of bacilli into several distinct species is probably based upon a misconception, and that all the forms which have been described under so many different names are but variants of a single species, *B. diphtheriae*, which constitutes the entire group.

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